

seropositive dogs had post therapy titer decreases (2 fold  $n=8$ , 4 fold or greater  $n=70$ ) as did all 3 people with CSD (4 fold or greater).

**Conclusion:** Antibiotic therapy of *Bartonella*-seropositive cats, dogs, and people can be effectively monitored using a comparative WB titration test.

doi:[10.1016/j.ijid.2008.05.1318](https://doi.org/10.1016/j.ijid.2008.05.1318)

70.021

#### Development of a Slide Latex Agglutination Assay for Identification and Confirmation of *Burkholderia pseudomallei* from Cultures

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**Keywords:** Latex particles; Monoclonal antibody; Sensitivity; specificity

Culture and biochemical assay remain as "gold standard" for identification and confirmation of *B. pseudomallei*. However confirmation by API 20NE biochemical assay is time consuming as the identification can only be done 24 hours onwards up to a week. Consequently, treatment of the patients suspected for melioidosis can be delayed and increases mortality rate as death can occur within 24 hours onset of the disease. Therefore there is a need to develop an assay that can shorten the time for identification and confirmation of *B. pseudomallei* from cultures. In this study, a rapid slide agglutination assay was developed using a specific monoclonal antibody raised against culture filtrate antigen of *B. pseudomallei* in Balb-C mice. The purified monoclonal antibody was tagged onto 0.8  $\mu$ m latex particles by means of passive adsorption. Latex agglutination assay was performed on a total of 45 *B. pseudomallei* strains and additional of five other *Burkholderia* species on glass microscope slide. Cross reactivity studies were performed on control panel strains which consisted of various gram-negative and gram-positive organisms. The latex agglutination assay was able to identify correctly 44 of 45 *B. pseudomallei* strains with a remarkable sensitivity of 97.8% and specificity of 100%. No cross reaction was observed with the control panel strains. In addition, latex agglutination assay was also performed on various *B. pseudomallei* antigen and compared with the developed assay. It was found that the developed latex agglutination assay is useful in identification and confirmation of *B. pseudomallei* from cultures.

doi:[10.1016/j.ijid.2008.05.1319](https://doi.org/10.1016/j.ijid.2008.05.1319)

70.022

#### Pneumocystis jiroveci Pneumonia (PCP) Diagnosis by Direct Fluorescent Antigen Detection

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**Background:** To compare the results of Direct Fluorescent antigen detection (DFA) with the routine direct examina-

(PCP) in bronchoalveolar lavage specimens (BAL) taken from symptomatic HIV seropositive patients.

**Methods:** Bronchoalveolar lavage specimens were examined from 57 symptomatic HIV seropositive patients sending to National institute of Health, Thailand. Specimens were examined for PCP using routine direct examination as Giemsa stain, Toluidine blue O stain and Ammoniacal silver stain and by Direct Fluorescent antigen detection (DFA)

**Results:** Of the 57 specimens analysed were 24 positive by DFA. An addition two specimens were routine direct examination negative and DFA positive. Compared to routine direct examination, the sensitivity and specificity of the DFA assay for the detection of *P. jiroveci* from BAL specimens sensitivity, specificity of were 100 and 94.3%. By the way positive predictive value (PPV), negative predictive value (NPV) and efficiency were 91.7 and 100 and 96.5%, respectively

**Conclusions:** this study has shown that DFA assay for the detection of *P. jiroveci* has a high sensitivity and specificity and can be adapted for use in the clinical microbiology laboratory require fluorescent microscopy.

doi:[10.1016/j.ijid.2008.05.1320](https://doi.org/10.1016/j.ijid.2008.05.1320)

70.023

#### Construction of the Internal Controls Using the Same Primers of the Target to Verify PCR and QPCR Results for Diagnosis

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**Background:** Current PCR and qPCR methods used in detecting and/or quantifying pathogenic agents present in patient sample have become the essential application for most diagnostic laboratories. Despite these methods have high sensitivity, technical issues are currently the most influencing factors for the quality of these present in-house methods. Therefore, development of a good quality control protocol has now become the vital next step.

**Objectives:** To develop and to construct the internal control using the same primers of the target in order to standardize the quality of PCR and qPCR results used in pathogen diagnosis.

**Methods:** Based on the molecular biotechnology, we designed and developed the internal controls which can be used with the same primers as for the target. After that, the LOD (limit of detection) concentration that can give detectable signal without inhibiting amplification of the target, were determined for these internal controls. These LOD determinations are very essential since the input of the internal control at the LOD to the samples and to the negative control sample can control the sensitivity of the nucleic extraction, the amplification; can detect the contamination as well as the inhibition without the need of multiple controls as usual

**Results:** Using the mentioned methods, various designed internal controls have been designed and constructed for